1,056,192

PATENT SPECIFICATION

NO DRAWINGS

1,056,192

Date of Application and filing Complete Specification: Jan. 8, 1965.

No. 977/65.

Two Applications made in France (Nos. 959,674 and 959,675) on Jan. 8, 1964.

Complete Specification Published: Jan. 25, 1967.

Index at acceptance:—A5 B(1H, 1L, 1Z, 2H, 2L, 2Z); C3 H3

Int. Cl.:—A 61 k 3/52

COMPLETE SPECIFICATION

Pharmaceutical Compositions containing Catalase

We, SINCOREP S.A., a Swiss Body Corporate of 24 Rue de Romont, Fribourg, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a process for the stabilisation of catalase and to pharmaceutical compositions containing stabilised catalase.

Catalase is an enzyme showing peroxidase activity which is widely distributed in nature and is found in larger or smaller quantities in virtually all human and animal tissues. It is of use in medicine in combatting such conditions as hypercholesteraemia and uricaemia.

Great difficulty is encountered in the therapeutic use of catalase, however, due to the fact that the enzyme has to be purified but is extremely instable in its purified form.

The catalase hitherto used in medicine has, in fact, been particularly sensitive to temperature changes. Besides being destroyed by heat like most enzyme substances, it is also sensitive to low temperatures; a rather exceptional circumstance. Catalase is also highly sensitive to the action of light among which the violet and ultraviolet fractions of the spectrum are the most destructive. Catalase is also sensitive to very many, even quite minor, chemical reagents and in particular it has been found that contact with glass, even with neutral glass, causes a progressive destruction of the enzyme activity.

Furthermore, experiments on preserving catalase by protection from changes of temperature (leaving the product between +5 and +10° Centigrade) and keeping it protected from any light, by storing it in neutral silicon flasks and in an atmosphere of an inert gas, have shown that the enzyme undergoes a degradation in spite of all these precautions, and causes a 50% drop of enzymatic activity in 45 to 60 days, dependent on the quantities present.

[*Price 4s. 6d.*]

Another very important fact is that the destruction of enzyme activity takes place not only when the catalase is stored in the form of a solution but also when it is stored in solid form. The sensitivity of the enzyme renders it difficult to make use of its therapeutic properties.

In fact, the sensitivity of the product to low temperatures, which is more clearly marked at about −20° C, makes the lyophilisation of the material, a process which otherwise appears to be most suitable for the preservation of drugs of this type, highly critical.

Furthermore, the sensitivity to light forces all manufacturing operations (dissolution, distribution, lyophilisation, corking, capping, labelling and placing into boxes) to be carried out in the dark or in a very weak red light, which fact makes it necessary to use special premises and work is made laborious and its quality suffers from the fact that the technicians work "blind."

Furthermore, since pharmaceutical legislation in certain countries, prohibits the use of opaque glassware, it is often impossible to protect the catalase from the destructive action of light at the moment of therapeutic use. Warnings that the vial should be used as soon as it is taken out of the box and that to close the boxes immediately after use, are not always followed strictly.

It is an object of the present invention to reduce the above disadvantages and to provide a process which allows one to stabilise catalase against the factors destroying its enzymatic activity.

According to the present invention we provide a process for the stabilisation of catalase, in which a hexol is added to the catalase as stabiliser. The hexol may be mixed with the catalase at any desired stage of the preparation thereof and is preferably used in the form of a solution.

According to a further feature of the invention we provide a pharmaceutical composition, for use in the treatment of arthrosis,

hepatitis, lipomatosis, uricaemia and hypercholesterolaemia, containing or consisting of catalase stabilised by a hexol as active substance.

In such compositions, the active substance is associated advantageously with a physiologically compatible carrier. The catalase is preferably in lyophilised form.

The catalase used in the therapeutic composition is stabilised by the addition of hexol during its isolation or purification and a certain quantity of this hexol remains with the catalase in the active substance used in the pharmaceutical composition, thus forming a composition which has, on the one hand, a very great stability *in vitro* to the usual agents destroying the enzymatic activity of catalase and, on the other hand, a greater stability *in vivo*, due to which the active substance has, as will be seen hereafter, therapeutic properties which have not been known previously for catalase alone, while fully preserving the known therapeutic properties of catalase. Other features of the invention will become apparent from the description which follows.

As is known, the preparation of catalase for therapeutic applications usually comprises several stages, for example: —

1. the extraction of crude catalase from an animal organ, such as the liver;

2. preparation of purified catalase from the crude catalase;

3. preparation of an aqueous solution of purified catalase and, if desired, lyophilisation of this solution in order to obtain a lyophilised material or crystallisation from said solution.

Since it is convenient, above all, to stabilise the catalase in a form which may be used in medicine, it is sufficient to add the hexol in the course of preparation of the aqueous solution of purified catalase, the latter being capable of being prepared, for example, by the conventional technique. A satisfactory stabilisation will thus be obtained either of the solution itself with the object of direct use, or of the lyophilised or crystallised catalase which will be prepared from this hexol-containing solution. The invention thus includes the addition of the hexol at this stage.

However, we have found that it is advantageous to introduce the hexol at an earlier stage of the preparation of the catalase and, preferably, at the first stage of the extraction of crude catalse from the animal tissue. If one works in this manner, for example, by effecting the extraction with an aqueous solution containing a hexol, a yield of crude catalase is obtained which is much higher than by the normal extraction with water without hexol. The yield can, in certain cases, be doubled or even more than doubled. This is probably due to the fact that the stabiliser prevents the destruction of an important part of this enzyme which is usual during this first extraction.

If one works in this way, it is necessary to ensure that during the later successive operations in the isolation of the catalase there is available a sufficient quantity of hexol to ensure the stabilisation of the catalase finally present and it may be necessary to add hexol during these later stages in order to ensure a sufficient hexol content in the final form of the catalase (solution, crystals or lyophilised powder).

In a preferred method of carrying out the process the extraction of the crude catalase from animal tissues is carried out by means of an aqueous solution of a hexol, the catalase being precipitated and then isolated from said solution, the crude catalase being dissolved in a second aqueous hexol solution, said second solution being dialysed while maintaining the content of hexol in the solution at a stabilising value, purified catalase being caused or allowed to precipitate from said second solution and dissolved in a third aqueous hexol solution, said third solution being lyophilised in order to yield a stabilised lyophilised catalase.

The quantity of hexol which is necessary for ensuring good stability of the catalase against destructive factors varies according to the particular hexol used; in general it varies between 5 g. and 50 g. of hexol per litre of a solution containing 100 millions of Beers and Sizer units (as defined in J. Biol. Chem. 1952, 195 page 133; one unit corresponds to one micromole of hydrogen peroxide decomposed per minute under standard conditions) of enzyme which corresponds to about 50 mg. to 500 mg. of hexol per million units of catalase. A content of 100 to 200 mg. of stabiliser per million units in the solution usually gives very satisfactory results. Since the stabilising power varies somewhat from one hexol to another, it is necessary to modify the quantities of the latter in accordance with this power. Quantities higher than those indicated above are usable but are not of great practical interest. The upper limit should take into account the toxicity of the hexol used.

The hexols are aliphatic compounds having six hydroxyl groups and those used as stabilisers according to the present invention preferably possess six carbon atoms; those originating from the reduction of sugars, for example mannitol, dulcitol, sorbitol or inositol are especially preferred. Mannitol is particularly interesting on account of its strong stabilising action and its absence of toxicity.

The presence of hexol in the stabilised catalase can easily be revealed by characteristic chemical reactions which are not necessarily the same for all usable hexols.

Two non-limiting examples of such characterisations are given hereafter by way of illustration only: all percentages are by weight: —

Test 1

Characterisation of mannitol, dulcitol and

sorbitol. A quantity of active substance containing 250,000 units of catalase activity is dissolved in 10 ml of water; the reaction solution is heated for five minutes on a boiling water bath. After cooling, it is filtered through paper and a solution A is obtained with which the following identification tests are carried out.

1. One ml of the following reagent I is added to one ml of solution A in a haemolysis tube. A white precipitate appears.

Reagent I		
HNO_3 (concentrated)		2 ml.
10% $AgNO_3$		2 ml.
2% KIO_4		25 ml.

2. To one ml of solution A, there is added one ml of periodic acid obtained thus:

Potassium periodate		4 g.
16% Sulphuric acid	q.s.p.	1000 ml.

One stirs and after leaving for five minutes there is added one ml of stannic chloride prepared thus:

Stannic chloride		23.5 g.
Fuming hydrochloric acid		50 ml.
Water	q.s.p.	1000 ml.

Two ml. of Schiff's reagent are then added. A pale blue coloration develops slowly. This coloration develops faster in the hot.

If the two reactions are positive, it is possible to conclude that mannitol, dulcitol or sorbitol is present in the active substance.

Test 2

Characterisation of inositol:

A quantity of active substance corresponding to 500,000 units of catalase are dissolved in 2 ml of fuming nitric acid. This solution is heated to dryness and a swollen-yellowish mass appears. The residue is taken up with 10 ml of distilled water and solution A is obtained.

1. Reaction with sodium nitroprusside:

Two drops of sodium hydroxide solution are added to 1 ml of solution A. A red coloration appears after gentle stirring. Five drops of an aqueous solution of freshly prepared 10% sodium nitroprusside are then added. The reaction solution is gently stirred and glacial acetic acid added up to acid reaction with litmus.

A blue-green coloration develops in the hot. After leaving for 1 hour, a blue precipitate is formed.

2. Reaction with barium acetate:

4 ml of a 5% solution of barium acetate are added to 2 ml. of solution A. After 2 minutes on a boiling water bath, a red coloration develops and a red precipitate is formed.

In order that the invention may be well understood the following Examples are given by way of illustration only:—

EXAMPLE I.

1 kg of homogenised ox liver is comminuted with one litre of a 1% aqueous solution of mannitol. Acetone is added in an amount corresponding to 4/10ths of the volume of the comminuted material. After filtering, the catalase is precipitated from the filtrate by adding slowly, and with continuous stirring, acetone corresponding to $\frac{1}{4}$ of the total volume. The product obtained, which is crude catalase containing a certain quantity of mannitol, is centrifuged and dried.

The crude catalase is redissolved in water containing 1% of mannitol, in a quantity such as to give a concentration of catalase of 100 million Beers and Sizer units per litre of solution. The insoluble material is removed and the supernatant liquid dialysed, whilst ensuring that the concentration of mannitol does not drop below 1% in the solution. More mannitol is added if necessary. After 48 hours of dialysis the catalase precipitates; it is redissolved in a buffer solution at pH 7.3 which is stored at 4° C. The catalase precipitates little by little in crystalline form. This stabilised, purfied catalase is then dissolved in a 1% aqueous solution of mannitol in the ratio of 100 million Beers and Sizer units per litre of solution and lyophilised. The active substance according to the invention is thus obtained in lyophilised form.

In order to illustrate the properties of stability *in vitro* of the active substance, a number of experiments have been carried out, in which it is subjected to the action of different catalase-destructive factors. Some of these experiments are described in the following examples.

EXAMPLE 2.

Aqueous solutions of the active substance were prepared, which contained 200,000 Beers and Sizer units of catalase and 1% by weight of mannitol. The stability was examined first by keeping the flasks at a temperature of 5° C, protected from light, and in non-silicon glass flasks. The results are given in the following Table I:

Times	Control Solution: units of catalase	Mannitol: units of catalase
0	200,000	200,000
12 hours	172,000	200,000
24 hours	126,000	200,000
48 hours	96,000	200,000
72 hours	82,000	200,000
4 days	79,000	200,000
5 days	76,000	200,000
6 days	71,000	200,000
7 days	68,000	198,000
9 days	64,000	198,000
11 days	63,000	195,000
13 days	61,000	193,000
15 days	57,000	193,000

The control solution only contained purified catalase without mannitol.

The table thus demonstrates that the control solution shows a loss of concentration of more than 50% in 48 hours and the solution of the active substance (catalase with mannitol) maintains a concentration which is invariable during 6 days but then shows a slow and progressive decline of concentration.

At another time, the same test was carried out while exposing the flasks to light. The losses of concentration were even more rapid for the control flasks but similar to the preceding ones for the flasks containing the active substance.

EXAMPLE 3.

The stability of the lyophilised active substance was examined.

Two solutions containing 100,000 Beers and Sizer units of catalase per ml. were lyophilised, one containing 1% of mannitol and the other containing no mannitol, distributing 0.25 ml. to each flask; the following results being obtained:

TABLE II

After lyophilisation	Catalase content per flask: Beers and Sizer units	
	Catalase only	Catalase with mannitol
immediately after	21,600	25,000
1 week after	18,200	25,000
2 weeks after	17,600	25,000
1 month after	13,400	25,000
2 months after	11,800	25,000
6 months after	8,200	25,000
12 months after	6,100	25,000

This experiment was carried out while keeping the flasks at a constant temperature of 5° C and protecting them from light.

EXAMPLE 4.

Flasks containing solutions of active substance were prepared as in Example 3.

The flasks were kept at ambient temperature which, in the course of this experiment, varied between 18 and 30° C, and a slightly quicker loss of the concentration was found in the control flasks, whilst the preservation of the bottles with active substance was as good as that in the course of the experiment of Example 3.

EXAMPLE 5.

Flasks containing solutions of active substance were prepared as in Example 3 and the action of light on the active substance, contained in the flasks was studied.

Table III gives the results obtained:

TABLE III

	Content per flask: units	
	Catalase only	Catalase with mannitol
Prior to the start of the experiment	21,900	25,000
after 1 hour	20,600	25,000
after 2 hours	18,700	25,000
after 4 hours	12,200	25,000
after 8 hours	8,100	25,000

The exposure of the flasks was carried out in normal daylight in well-lighted premises on a cloudless day. Photo-electric cells made it possible to verify that all flasks received the same illumination.

From the foregoing and other tests carried out, it can be seen that the process of the invention, results in

—excellent preservation of solutions of stabilised catalase for at least 6 days, both in daylight as well as while protected from light;

—the possibility of proceeding with the operations of lyophilisation under normal light without substantial loss of concentration;

—the possibility of preserving for at least 12 months lyophilised flasks without a substantial decline of concentration, whilst bottles with an identical lyophilised catalase without a stabiliser show, under the same condition, a loss of concentration of more than 70%;

—the possibility of exposing the lyophilised preparation to daylight for at least 8 hours without a substantial loss of concentration.

Several results of toxilogical tests carried out with stabilised catalase (mannitol being the hexol) are given hereafter:

I—*Acute toxicity*:

a) on the mouse: doses corresponding to 2,400,000 units of catalase activity/kg body weight administered by the peritoneal route did not give rise to symptoms of toxicity.

b) on the rat: the active substance was administered by the intramuscular route at a dose of 1,500,000 units of catalase activity/kg body weight. The animals did not show any visible disorder.

c) on the rabbit:

1) by the intravenous route: a dose of 20,000 units/kg body weight was readily tolerated,

2) by the rectal route: also a dose of 600,000 units/kg body weight was readily tolerated by the animal under experiment,

3) perorally: a dose of 3,500,000 units/Kg body weight did not induce any toxic symptoms.

II—*Chronic toxicity*:

a) on the guineapig:

—by the intramuscular route: a dose of 25,000 units per guineapig of about 400 g., administered every day for 70 days, was readily tolerated.

b) on the mouse; a daily dose of 1,200,000 units/kg body weight administered for 60 days by the intramuscular route, was readily tolerated;

c) on the rabbit:

1) by the intramuscular route: a dose of 50,000 units/kg body weight administered for 80 days, was readily tolerated;

2) by the rectal route: a dose of 100,000 units/kg body weight was readily tolerated for 70 days.

3) perorally: a dose of 120,000 units/kg body weight administered for 92 days, was readily tolerated.

III—*Local and general tolerance*

The local and general tolerance of the active substance, both in the examination of acute toxicity, as well as in that of chronic toxicity, proved itself very satisfactory.

The pharmacological properties of stabilised catalase are almost identical with those of catalase alone.

The therapeutic composition of the invention is usable not only in the known applications of catalase, such as the treatment of hypercholesterolaemia and uricaemia for example, but it also has novel therapeutic properties which the known catalase does not possess. New applications include the treatment of arthrosis, hepatitis and lipomatosis.

The composition may be administered, for example, by the parenteral or rectal routes in doses which, taking into account the absence of toxicity of the active substance, vary widely according to the case to be treated.

It can be presented, for example, in the form of injectable solutions, formulated in ampoules each containing 25,000 Beers and Sizer catalase units or in suppositories each containing 200,000 Beers and Sizer catalase units, the active substance being associated with appropriate carriers in their pharmaceutical forms.

In order to illustrate the therapeutic properties of the composition of the invention, several clinical observations (the pharmaceutical forms and unitary doses are those described above) will be given hereafter by way of examples.

I—Disturbances of the metabolism of uric acid:

1st observation: Mrs. Celine GR.—65 years
—clinical diagnosis: praecordial pains, — buzzing in the ears, hypercholesterolaemia, hyperuricaemia;
—treatment with the composition of the invention in injectable form. 41 intramuscular injections in 5 months.
Tolerance: excellent. A marked functional improvement.
—Biological balance sheet:
Prior to treatment: cholesterol=3.50 g/1000 ml serum. Total lipids: 9.60 g/1000 ml serum; uricaemia=71 mg/1000 ml serum.
After the treatment: cholesterol=3.22 g/1000 ml serum. Total lipids: 8.30 g/1000 ml serum; uricaemia: 54 mg/1000 ml serum.
Conclusion: Remarkable functional improvement. Good biological results.

2nd Observation: Mr. Georges FR.—52 years
—Clinical diagnosis: gout.
—Treatment with the composition of the invention: 30 intramuscular injections in two months, and then 20 suppositories during one month.
—Biological balance sheet:
Prior to treatment: uricaemia: 120 mg/1000 ml serum.
After the treatment by injections uricaemia: 72 mg/1000 ml serum.
After the rectal treatment: uricaemia: 45 mg/1000 kg serum.
—Conclusion: excellent tolerance—suppression of gout crises—a good biological improvement.

3rd Observation: Mrs. Rolande PI.—58 years
—Diagnosis: myocarditis associated with hyperuricaemia.
—Treatment: 47 intramuscular injections of the composition of the invention in 2 months. Excellent tolerance.
—Biological balance sheet:
Prior to treatment: cholesterol: 4.00 g/1000 ml serum, uricaemia: 100 mg/1000 ml serum.
After the treatment: cholesterol: 3.40 g/1000 ml serum, uricaemia: 65 mg/1000 ml serum.
The rate of uricaemia is maintained for 4 months due to a rectal treatment (10 suppositories per month).
—Conclusion: a good subjective result, a good biological result.

II—Viral icterogeneous hepatitis:

1st Observation: Mr. Melchior B—22 years
—Diagnosis: icterogeneous hepatitis.
—Treatment: 2 injections of the composition per day, for 15 days.
—Biological balance sheet:
Prior to treatment: bilirubinaemia: 64 mg/1000 mg serum; Mac Legan: 40 Vemes photometric units; blood urea: 0.44 g/1000 ml serum; cholesterol: 1.45 g/1000 ml serum.
After the treatment: bilirubinaemia: 5 mg/1000 ml serum; Mac Legan: 24 Vemes photometric units; blood urea: 0.47 g/1000 ml serum; cholesterol: 2.05 g/1000 ml serum.
—Conclusion: tolerance perfect.
A very good clinical result: after one week of treatment, disappearance of the asthaenia, and an almost complete disappearance of the icterus. After one month of rest: the patient is clinically healed.

2nd Observation: Mr. Jacques S.—27 years
—Diagnosis: icterigeneous hepatitis contracted during the subsidence of an influenzial syndrome.
—Treatment: 2 injections of the composition per day for 28 days—a series of 10 suppositories during the following month.

—Biological balance sheet:
Prior to treatment: bilirubinaemia: 100 mg/1000 ml serum; Mac Legan: 72 Vemes photometric units; blood urea: 0.24 g/1000 ml serum; cholesterol: 1.65.
After the treatment: bilirubinaemia: 10 mg/1000 ml serum; Mac Legan: 18 Vemes photometric units; blood urea: 0.30 g/1000 ml serum; cholesterol: 2.35 g/1000 ml serum.

III—Arthrosis:
1st Observation: Mr. Marius A.—50 years
—Diagnosis: strongly developed bilateral coxarthrosis.
—Treatment: 30 injections of the composition in 50 days plus medical orthopaedy.
—Results: a clear improvement of the pain phenomena and of the functional importance —tolerance excellent.
2nd Observation: Miss Yvonne L.—38 years.
—Diagnosis: dorsolumbar stroke, overweight.
—Treatment: 25 injections in 40 days plus orthopaedic hygiene.
—Results: good for the pain phenomena—tolerance perfect.
3rd Observation: Mrs. Marie-Louise D.—48 years
—Diagnosis: light gonarthrosis, bilateral coxarthrosis, discarthrosis between lumbar vertebrae L_3—L_4 and L_4—L_5 and between lumbar vertebra L_5 and sacral vertebra S_1.
—Treatment: 20 injections in 30 days plus 20 suppositories in 20 days.
—Improvement of the lumbar pains and of the pains in the knees. Tolerance perfect.

IV—Lipomatoses:
—*Observation*: Mr. Jean PO—60 years
Diagnosis: Launois-Bensaude's lipomatosis.
—Treatment: one injection of the composition per day for 6 months.
—Biological balance sheet:
Prior to treatment: cholesterol: 1.75 g/1000 ml serum
α-lipoproteins: 16.3%
β-lipoproteins: 83.7%
After the treatment: cholesterol: 1.90 g/1000 ml serum.
α-lipoproteins: 12.8%
β-lipoproteins: 87.2%
—Clinical results: very good. Softening of the lipomatous masses which have decreased in volume and some of which disappeared. Tolerance excellent.
Results maintained by treatment with 10 suppositories per month, every month.

WHAT WE CLAIM IS:

1. A process for the stabilisation of catalase, in which a hexol is added to the catalase as stabiliser.

2. A process as claimed in claim 1, in which said hexol is added to the catalase at any stage of the isolation or purification thereof.

3. A process as claimed in claim 1 or claim 2, in which the hexol is added to the catalase in the form of an aqueous solution.

4. A process as claimed in any of claims 1—3, in which the hexol contains 6 carbon atoms.

5. A process as claimed in claim 4 in which the hexol is mannitol.

6. A process as claimed in claim 4 in which the hexol is dulcitol, sorbitol or inositol.

7. A process as claimed in claim 3 in which the catalase is dissolved in an aqueous hexol solution.

8. A process as claimed in any of the preceding claims in which said catalase is a purified catalase free from stabilisers.

9. A process as claimed in any of claims 1—7 in which said catalase is a purified catalase which has been stabilised beforehand by a hexol during its preparation.

10. A process as claimed in any of the preceding claims in which an aqueous solution of purified catalase, containing said hexol, is lyophilised.

11. A process as claimed in any of the preceding claims in which 50 to 500 mg. of hexol are used per million Beers and Sizer catalase units (as herein defined).

12. A process as claimed in claim 11 in which 100 to 200 mg. of hexol are used per million of said catalase units.

13. A process as claimed in any of the preceding claims in which the extraction of the crude catalase from animal tissues is carried out by means of an aqueous solution of a hexol, the catalase being precipitated and then isolated from said solution, the crude catalase being dissolved in a second aqueous hexol solution, said second solution being dialysed while maintaining the content of hexol in the solution at a stabilising value, purified catalase being caused or allowed to precipitate from said second solution and dissolved in a third aqueous hexol solution, said third solution being lyophilised in order to yield a stabilised lyophilised catalase.

14. A process as claimed in claim 1 substantially as herein described.

15. A process as claimed in claim 1 substantially as herein described with reference to Example 1.

16. A pharmaceutical composition, of use in the treatment of arthrosis, hepatitis, lipomatosis, uricaemia and hypercholesterolaemia, containing or consisting of catalase stabilised by a hexol as active substance.

17. A composition as claimed in claim 16 in which the active substance is present in lyophilised form.

18. A composition as claimed in claim 16 or claim 17 in which the hexol contains 6 carbon atoms.

19. A composition as claimed in claim 18 in which the hexol is derived from a sugar.

20. A composition as claimed in claim 19 in which the hexol is mannitol.

21. A composition as claimed in claim 18 in which the hexol is dulcitol, sorbitol, or inositol.

22. A composition as claimed in any of claims 16—21 in which the quantity of hexol in the active substance, is between 50 and 500 mg. per million of Beers and Sizer catalase units (as herein defined).

23. A composition as claimed in any of claims 16—22 in a form suitable for administration by the intramuscular or by the rectal route.

24. A composition as claimed in any of claims 16—23 in dosage unit form.

25. A composition as claimed in any of claims 16—23 in the form of injectable solutions or of suppositories.

26. A composition as claimed in any of claims 16—25 in which the active substance is associated with one or more pharmaceutical carriers or excipients.

27. A composition as claimed in claim 16 substantially as herein described.

28. Catalase stabilised by a hexol whenever prepared by a process as claimed in any of claims 1—15.

For the Applicants,
FRANK B. DEHN & CO.,
Chartered Patent Agents,
Imperial House, 15—19, Kingsway,
London, W.C.2.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press (Leamington) Ltd.—1967. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2, from which copies may be obtained.